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Magnetic Field Effect on Lysozyme Crystalized by LLIP Method II

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The Liquid-liquid interfacial precipitation (LLIP) method is one of crystallization methods. We reported previously the size of lysozyme crystals was increased when it was crystallized and levitated under vertical gradient magnetic field using a paramagnetic salt as a precipitant [1]. However, the size was increased even under horizontal gradient magnetic field without magnetic levitation [2]. In this paper, we crystallized lysozyme under various horizontal gradient magnetic fields, and the magnetic field effects were investigated.

The 100 μl precipitant solution including PEG4000, $CoCl_2$ and TAE buffer was poured into a reactor with 6.5 mm diameter. The 50 μl protein solution including lysozyme and TAE buffer was stacked gently to make the interface between them. In addition, 100 μl liquid paraffin was stacked on the protein solution to prevent evaporation of the solutions. The reactor was set under horizontal magnetic field (BdB/dz = 0, 104, 202, 301, 416 T^2/m) at 20 °C for 24h.

The crystals precipitated under gradient magnetic field were observed at position away from the center of the magnetic field, and the size was increased. The reactor was divided into three zones, and the sizes of crystals obtained in each zone were plotted in Fig. 1. In Zone 3, the size of the crystal increased as the magnetic force field increased. In order to confirm the interface at each magnetic force field, *in situ* observation was conducted. Fig. 2 shows the magnetic force field dependence of the angle θ between the protein-precipitant interface and the horizontal direction, and the average length of *a*-axis obtained in Zone 3. The angle of the interface was saturated at BdB/dz = 250 T^2/m. On the other hand, the crystal size increased thereafter. This is probably because the magnetic force applied to the crystal continues to increase even after the angle was saturated. As the magnetic force increases, the growth position of the crystal, concentration distribution and the convection were changed. It is thought these factors also cause the size increase of the crystal after saturation of the angle of the interface.

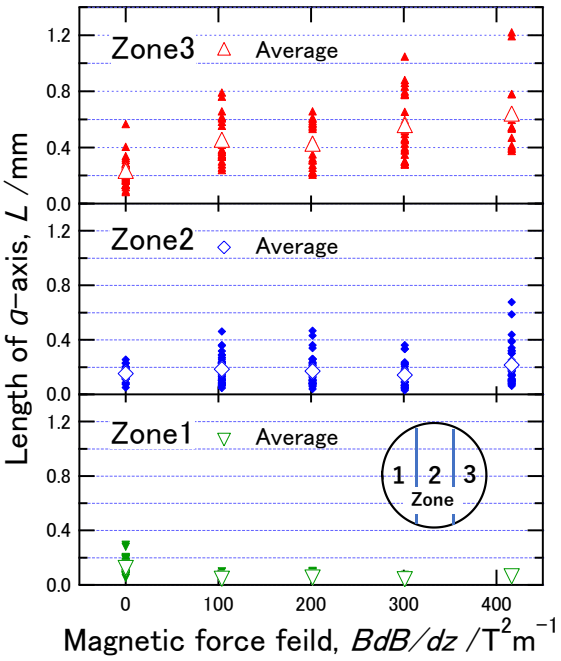

Fig. 1. The size distribution of lysozyme crystals precipitated under horizontal magnetic field.

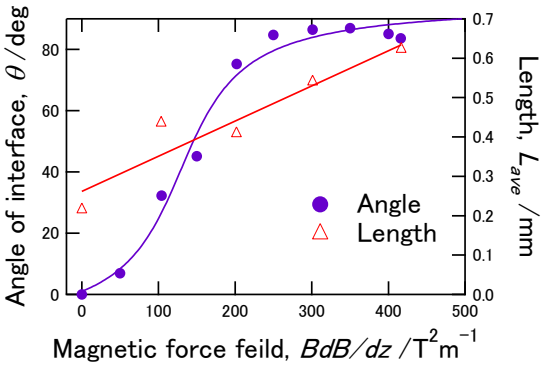

Fig. 2. The magnetic force field dependence of the angle of protein-precipitant interface, and the average length of *a*-axis in Zone 3.

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References: [1] T. Onotou, *et al.*, The 64th JSAP Spring Meeting, 14p-P10-96.
[2] T. Okabe, *et al.*, The 65th JSAP Spring Meeting, 19a-A202-2.